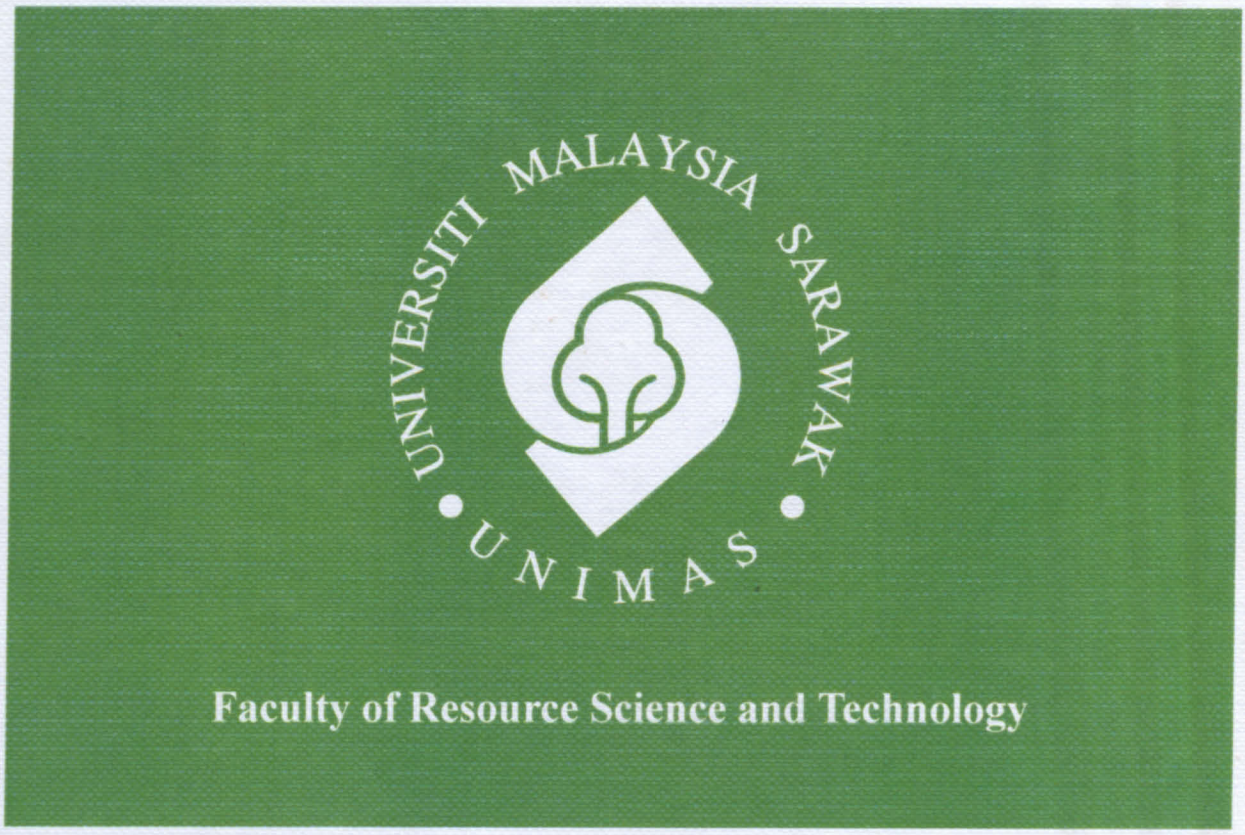

2D-PAGE Analysis of Chicken Samples from Different Slaughtering Methods

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(50819)

Bachelor of Science with Honours
(Resource Biotechnology)
2018

2D PAGE analysis of chicken samples from different slaughtering process

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A thesis report submitted in partial fulfilment of Final Year Project (STF3013) Resource Biotechnology

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7 May 2018

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2D-PAGE Analysis of Chicken Muscle from Different Slaughtering Methods

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Abstract

This study investigated the expression of protein in chicken meat that assumed to be influenced by variations of the method of slaughtering. There were two types of samples that depend on the method of slaughtering had been examined. Samples are: (i) Severing the trachea, oesophagus and carotid arteries and jugular veins which are halal method, and (ii) Cut directly the neck of the chicken without considering the veins which are non halal method. Proteins in the sample had been extracted by two different extraction methods and quantified with Bradford assay to know the protein concentration. Protein expressed in higher amounts mostly found distributed at range of Mw 63-100 kDa for direct extraction method, meanwhile for TCA extraction method protein expressed highly at range of Mw 50-75 kDa as resolved using SDS-PAGE. Through 2D-PAGE, protein spotted on the gel highly expressed at range of Mw 8 kDa and 30 kDa for halal chicken samples but not highly expressed for non-halal chicken samples.

Keywords: Protein; Halal; Non-Halal; SDS-PAGE and 2D-PAGE

Abstrak

Kajian ini menyelidik ungkapan protein dalam daging ayam yang diandaikan dipengaruhi oleh variasi kaedah penyembelihan. Terdapat dua jenis sampel yang bergantung kepada kaedah penyembelihan telah diperiksa. Sampel adalah: (i) Memotong arteri trakea, esofagus dan karotid serta urat jugular yang merupakan kaedah halal, dan (ii) Memotong leher ayam secara langsung tanpa mempertimbangkan urat yang merupakan kaedah tidak halal. Protein dalam sampel telah diekstrak oleh dua kaedah pengekstrakan yang berbeza dan diteruskan dengan ujian Bradford untuk mengetahui kepekatan protein. Kandungan protein didapati tinggi dalam taburan Mw 63-100 kDa untuk kaedah pengekstrakan langsung manakala Mw 50-75 kDa untuk kaedah pengekstrakan TCA dengan menggunakan SDS-PAGE. Sementara itu, melalui 2D-PAGE tompok protein kelihatan pada gel didapati tinggi sekitar Mw 8 kDa and 30 kDa telah dikesan pada ayam yang disembelih secara halal tetapi tidak pada ayam yang disembelih secara tidak halal.

Kata Kunci: Protein; Halal; Tidak Halal; SDS-PAGE dan 2D-PAGE

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List of Abbreviations

APS	Ammonium Persulfate
CBB	Coomassie Brilliant Blue
CP	Control Point
DTT	Dithiothreitol
IPG	Immobilized pH Gradient
IEF	Isoelectric Focusing

1.0 Introduction

1.1 Background

Islam is the world's second largest religion. Islam is not only a religion of rituals but also a way of life (Addeen, 2014). In Islam, eating considered as a matter of worshipping God, like ritual prayers. Islam law prescribes a set of dietary rule called Halal that lists the permitted food and prohibit consumption of meat that not obtained according Islamic rules that cover livestock handling before and after slaughter (Regenstein et al., 2003; Bonne, 2008). Muslims must follow Islamic dietary rule and the foods must meet that rule called Halal. Muslims need to make an effort to obtain Halal food as it is religious obligation to consume halal food. For non-Muslim consumers, halal foods are often perceived as specially selected and processed to achieve Halal standards of quality (Addeen, 2014).

Poultry production involves series of interrelated steps that been designed to convert domestic birds to ready-to-cook whole carcasses, cut up carcass parts or various forms of deboned meat product (Alan, 2001). Food safety and the shelf-life aspects of chicken meat are important concerns in relation of microbial growth (Addeen, 2014). Focus is mainly on the absence or present of potentially pathogenic microbes such as *Salmonella spp.* and *Campylobacter spp.* (Ali et al., 2011). Most important factor that affect level of contamination and enhance the extent of deterioration is the amount of blood that left within the carcass after bleeding (Ali et al., 2011). Blood is considered as an excellent medium for growth of bacteria due to high nutritive value, temperature, pH and relative humidity (Addeen ,2014). Amount of blood bled by animal depends on the slaughtering method (Ali et al., 2011). Blood components such as haemoglobin are powerful promoters of lipid oxidation and could decrease shelf-life of meat products.

There are differences in slaughtering methods practiced over the world. Different methods of poultry slaughtering are approved and practiced based on common aspects. Slaughtering methods that been practiced in the world include the Halal method, hanging method and stunning method (Addeen, 2014). The Islamic method is traditional method of slaughtering for Halal foods. Stunning prior to slaughtering is not permitted and if the stunner's voltage is too high, chicken would be dead before slaughtering. According to Sams (2001), harsher electrical stunning results in higher incidence of haemorrhaging and broken bones which make the chicken meat be haram (Directorate of Veterinary Public Health, 2006). Chickens that dead before slaughtering would cause incomplete bleeding process. Halal slaughtering accomplished by a throat cut that would bring the animal to a quick death without suffering by the reaction of carotid arteries, jugular veins, trachea and oesophagus and also absence of previous stunning, that allow rapid and complete bleeding (Grandin and Regenstain, 1994). Slaughtering process for chicken can be varied as it depends on the belief or practice, however, Halal method been believes to render complete bleeding and may be beneficial for shelf-life extension or quality maintenance of chicken meat (Addeen, 2014).

1.2 Hypothesis

Severance of neck and different degree of muscle movement would influence release and/or retention of related biomolecules in consumable parts of slaughtered animals. It has been hypothesized that different slaughtering methods could be identified by the resulting of different protein profile.

1.3 Objective

The current study help to assess difference protein profile content in muscle sample in chicken slaughtered by different slaughtering methods and also to observe protein expression of chicken samples that slaughtered by different slaughtering methods.

2.0 Literature Review

2.1 Poultry Slaughtering

Number of poultry meat production may vary in different parts in this world but the importance of this sector remains the same (Passantino, 2009). Examples of poultry meat from different species of domestic birds such as chicken, turkey, geese, duck etc. However, the dominant production type is chicken (Passantino, 2009). Poultry production and processing involve a series of interrelated steps that designed to convert domestic birds into ready to cook whole carcasses, cut up parts or forms of deboned meat products (Alan, 2001).

Quality production of poultry meat requires clean, safe and humane handling of the poultry animals (Zaman, 2012). Good poultry slaughtering process is the one that fulfil hygienic-sanitation requirement (Adden, 2014). Most developed countries have humane slaughter laws for food animals that cover poultry species. The laws designed help to ensure animals are killed quickly, painlessly without suffering (Raj, 1998). Guidelines based on halal and kosher principles vary from guidelines of slaughtering according region. For small scale guidelines, strictly followed cutting both jugular veins and carotid arteries without stunning (Zaman, 2012). However, stunning can also applied with restriction (Zaman, 2012).

2.1.1 Stunning slaughtering process

Stunning before slaughter is a requirement in Europe and performed to induce unconsciousness and insensibility in animals so that slaughter can be performed without causing animals any anxiety, pain, suffering or distress (Terlouw et. al., 2008). The stunning techniques are part of slaughter procedure and types of stunning used depends on species as stated in Table 2.1. As can be seen in Table 2.1, stunning methods for poultry are electrical, mechanical and gas mixture (Terlouw et. al., 2008).

Table 2.1: Stunning methods used for different species.

Species of Animals	Generally used stunning methods in Europe
Cattles and calves	Mechanical
	Electrical
Sheep	Mechanical
	Electrical
Pigs	Mechanical
	Electrical
	Gas Mixtures
Poultry	Electrical
	Mechanical
	Gas Mixtures
Fish	Manual
	Thermal
	Electrical

The most common and simplest stunning method is electric shock that known as electrical stunning (ES) system which initially developed to immobilize the bird long enough to allow physical manipulation of bird for alignment in neck cutting equipment and reduce carcass damage that happen due to unconscious physical activity such as wing flapping (McNeal et al., 2003). Primary functions of stunning are to render animal unconscious for easier handling during slaughter and render animal insensible to pain (Addeen, 2014).

Gas stunning is an alternative method that been used by the European Economic Commission since it help to render the bird quickly (McNeal and Fletcher, 2003). After been stunned, usually general slaughter procedures used to exsanguinate the broiler (Addeen, 2014). Decapitation is another acceptable means of killing chickens that described by the American Veterinary Medical Association (1993). Decapitation after low-voltage electrical stunning (ES) system can be used as an alternative to high-voltage stunning that can cause meat quality problems such as haemorrhaging (Addeen, 2014). Decapitation also may cause higher pH at 24 and give no effect on color or tenderness if compared to other methpds (McNeal and Fletcher, 2003). According to McNeal et al. (2003), studies shown that decapitation can be used as an alternative to conventional ES method successfully and not result any negative effect on carcass and meat quality (McNeal and Fletcher, 2003).

2.1.2 Slaughtering process without stunning (Halal)

The OIE (2009), the EU and the USA have permit slaughtering without stunning to allow Jews and Muslims to practice their religious belief (Addeen, 2014). Some religious authorities will acceor stunning either before or after the throat cut which help in improving animal welfare (Addeen, 2014). Slaughtering by hand still preferred by all Muslims and widely followed in the Muslim countries and also countries that Muslims control their slaughterhouses (Sams, 2001).

Poultry processing plants where Halal and non-Halal birds are processed, halal birds need to be make sure completely segregated during chilling, processing and storing (Addeen, 2014). Further processing such as marinating and breading should be done under supervision of qualified halal inspector by using cleaned equipment (Addeen, 2014). The main argument against pre-slaughter stunning according to Muslim factions, the stunning process can hinder blood loss as stunning may changes the muscular, neurological and cardiovascular status of the animal (Anil et al., 2004). Slaughtering chicken without stunning actually more difficult than slaughtering stunned chicken (Addeen, 2014). Procedure for slaughtering without stunning is similar to the procedure of chicken slaughtering with stunning, butcher must have spare knifes to ensure the sharpness and cleanliness (Addeen, 2014). After slaughtering, chicken is placed into specific box or container that easy to clean up and during this procedure, it is a must to ensure blood flows perfectly for around three to five minutes or until chicken completely dies (Addeen, 2014). Halal method can cause severe pain to animal when small knife is used and numerous attempts are made to severe all the vessels in the neck (Grandin and Regenstein, 1994).

2.2 Halal Slaughtering of Poultry

Halal poultry meat production should meet industry, economic and production needs and health requirements which supposed to be without compromising Islamic religious requirements that been derived from the Quran and the Hadiths (Shahdan, 2016). Halal certification authorities may vary in their own interpretation of teachings that leads to differences in halal slaughter requirements (Shahdan, 2016). Animal-derived food ingredients like emulsifiers tallow and enzymes must be made from animals slaughtered by a Muslim to be Halal (Regenstein et. al., 2003). In Islamic law, Muslims are prohibited from eating the flesh of pork and its derivatives as these rulings have been stated from Islamic law as guidelines (Addeen, 2014). Besides that, eating of haram materials and used it as an adulterant or additive in food products are forbidden (fadzillah et al., 2011). Current study proposes 6 control points (CP) for halal poultry meat production based on the most commonly used halal production system (Terlouw et al., 2008).

CP 1 described what is allowed and prohibited such as blood and animal manure and feed ingredients for halal poultry meat production (Shahdan et. al., 2016). CP 2 help to describe requirement in humane handling during lairage meanwhile CP 3 describes different methods to immobilize poultry when immobilization is used (Shahdan et. al., 2016). CP 4 describes the importance of intention, details of the halal slaughter (Shahdan et. al., 2016). Lastly CP 5 and CP 6 help to describe requirements after the neck cut such as time needed before carcasses can enter scalding tank and potential for meat adulteration with faecal residues and blood (Shahdan et. al., 2016).

Demand for halal meat and meat products serves to challenge food industry to expand the production without have to compromise the halal integrity of product being certified (Farouk, 2013). Halal slaughtering without inducing unconsciousness has been alleged to be in humane compared to secular slaughter. Muslim have adopted electrical head only stunning (ES) (Nakgin-Sige et al., 2013) but the use of pre-slaughter ES prior to neck cut been linked to blood splash in a range of muscles of slaughtered animals (Hindle et. al., 2010).

Among the listed halal monitoring authorities in Table 2.2, only Australia and Malaysia have halal monitoring systems which overseen by their governments (Shahdan et. al., 2016). Malaysia's JAKIM monitors the halal integrity for both local and export products meanwhile the Australian government involved only with products for export (Shahdan et. al., 2016).

Table 2.2: Available guidelines from different countries or agencies for halal poultry slaughtering.

Country/ Agency	Halal monitoring authority/relevant guidelines	Available guidelines at various steps of poultry meat slaughtering and processing			
		Farm	Pre-S	S	Post-S
Australia	Australian Quarantine and Inspection Service (AQIS)	X	X	/	/
Arab Gulf	Arab Gulf Cooperation Council (GCC)/GCC Standard 993/2998	X	X	/	/
Indonesia	Indonesian Council of Ulama (MUI)	X	X	/	/
Malaysia	Department of Islamic Development Malaysia (JAKIM)	X	X	/	/
Singapore	Islamic Religious Council of Singapore (MUIS)	X	X	/	/
Thailand	Central Islamic Committee of Thailand (THS)	X	X	/	/
UK	Halal Monitoring Committee	X	/	/	/
US	Halal Food Standards Alliance of America	/	X	/	/
WTO	CODEX Alimentarius CAG/GL	X	X	/	X

2.2.1 Halal requirements

Classification of animals into halal or haram clearly stated in the Quran as the permission to kill animals quoted in religious texts (Addeen, 2014). Meat of the birds which do not use their claws to hold down food such as chicken, turkeys, ducks, geese and pigeons is permitted (Addeen, 2014). There are special requirements for slaughtering the animal which are, an animal must be Halal species, slaughtered by an adult and sane Muslim, Allah's name involved at the time of slaughter and slaughtering must be done by cutting the throat in manner that help to induce rapid and complete bleeding as it will result in quickest death (Addeen, 2014). Generally accepted method is by cutting at least three to four passages which are the carotids, jugular vein, trachea and oesophagus (Addeen, 2014).

Some Islamic scholars accept machine slaughter particularly in poultry but in recent years, the trend has gone back requiring hand slaughter of animals (Regenstein et al., 2003). Meat of slaughtered animals is called "zabiha" meat (Khan, 1991). Islam emphasis on gentle and humane treatment of animals specifically before and during slaughter (Ali et al., 2011). Islam encourages few conditions include giving animal proper rest and water, avoid conditions that would create stress and using a sharp knife to slit the throat (Addeen 2014). After the blood allowed to drain completely from the animal, only then that animal can further be subjected to the dismemberment; cutting off of horns and legs (Raiz and Chaudry, 2004). Unlike kosher, soaking and salting the carcass is not required for Halal (Raiz and Chaudry, 2004).

2.2.2 Conditions and Method of Islamic slaughtering

Dhabh defined as method of killing an animal for the purpose of making its meat to fit human consumption (Addeen, 2014). There are few conditions that need to be fulfilled in order for dhabh meet the requirements of Syariah (Khan, 1991).

(a) The slaughter person: Person that perform the act of dhabh must sound mind adult Muslim which can be either sex (Addeen, 2014). If that person lacks or lost the competence through intoxication, he or she may not perform Halal slaughter (Addeen, 2014).

(b) The instrument: Knife that been used to perform dhabh must extremely sharp as it will help to facilitate quick cutting of the skin and severe blood vessels to enable blood to flow quickly which will bring to a massive haemorrhage (Addeen, 2014). It is a tradition stated that not to sharpen the knife in front of the animal that about to be slaughtered (Raiz and Chaundry, 2004).

(c) The cut: Incision need to be made in the neck at certain point below the glottis and base of the neck (Addeen, 2014). According to Raiz and Chaundry (2004), process called nahr defined as spearing hollow of the neck but now with modern restraining and stunning methods, this process might not be appropriate anymore (Addeen, 2014). Trachea and oesophagus need to be cut in addition to the jugular veins and carotid arteries, spinal cord must not be cut and head therefore not to be severed completely (Addeen, 2014). Note that kosher method is very similar to traditional method of dhabh except for the invocation that not made on each animal (Khan, 1991).

2.3 Effect of slaughtering methods on the quality of poultry meat

2.3.1 Factors affecting bleeding at the time of slaughtering

Major factors that affect bleeding include the blood vessels severed, size and patency of sticking wound, orientation of carcass; horizontal or vertical, cardiac arrest and muscle contractions squeezing blood capillaries and vesséls (Gregory, 2005). Processing factors such as time to bleed and dressing procedures to allow blood to escape also affect the bleeding (Addeen , 2014). Others factors such as health, weight, breed, sex and stress were reported to affect bleeding in sheep (Khalid, 2011).

In broiler processing industry, high frequency currents result in birds with engorged wing veins and wing haemorrhages compare with the low frequencies which are 50 or 60 Hz (Addeen, 2014). According to Wilkins and Wotton (2002), recently confirmed for turkeys which found that the effects of high frequency electrical stunning on breast meat were fall in pH and quality considered to be minor. Bleeding efficiency is greater with high frequencies as the prevalence of cardiac arrest at stunning is lower (Mouchoniere et al., 1999).

According to McNeal et al. (2003), one way to reduce bloody pygostyles that unsightly blemish in poultry, instead of normal neck cut was by decapitating the birds after stunning. Decapitation after electrical stunning would result in an advantage where the body lost physical activity right after cutting (Addeen, 2014). Stunning only the head results in breast muscle haemorrhages at the humeruscoracoid joint and whole body stunning results in haemorrhages be in the middle of the muscle (Hillebrand et al., 1996).

2.3.2 Effect of slaughtering methods on bleeding and meat quality

Islamic hanging method is the best bleeding method compared with Islamic traditional slaughtering method and electric stunning method; therefore, it is recommend to be used for slaughtering of broiler chickens (Addeen, 2014). According to Ali et al. (2011), amount of blood collected after slaughtering of broiler chickens by using three different methods such as Islamic traditional, Islamic hanging and electric stunning methods were varied. Electrical stunning method showed the lowest weight of blood meanwhile slaughtered by Islamic hanging method had the highest weight of blood collected (Ali et al., 2011). Islamic hanging without stunning leads to more bleeding and could be attributed to effect of gravity and rapid speed of blood flow in blood vessels before clotting (Ali et al., 2011).

Electrical stunning been associated with a decrease in carcass blood loss (Papinaho and Fletcher, 1995) even though some exception had been reported by Dickens and Lyon (1993). According to Papinaho and Fletcher (1995), recent research suggest that electrical stunning only affects rate of early blood loss and give little effect on ultimate carcass blood loss. Meat that derived from animals slaughtered without stunning results in higher pH values, lower drip loss and some petechial hemorrhages (Dagata et al., 2009). Meanwhile, meat that derived from ritual slaughter had an unpleasant aspect because of small red spots on the surface.